ULTRASONOGRAPHY AND BIOCHEMICAL STUDIES OF HEPATOBILIARY SYSTEM IN BUFFALOES

Raveendra Tadagani, Rudra Pratap Pandey, Sanjay Purohit and Gulshan Kumar*

Received: 28 September 2020
Accepted: 18 June 2022

ABSTRACT

The present work was undertaken to study the ultrasonographic and clinico-biochemical parameters of hepatobiliary system in apparently healthy adult non gravid buffaloes. The present study revealed that, the hepatic parenchyma was homogenously coarse echogenic, interspersed with anechoic bands of hepatic vessels, with sharp margin and was hyperechoic relative to right renal cortex. It was imaged from just behind the last rib to the 6th intercostal space. The gallbladder was visualized between the 12th to 9th intercostal spaces, seen as a pear-shaped fluid-filled anechoic structure with hyperechoic wall, restricted to one to two intercostal spaces. The portal vein was seen as a stellate, branching, anechoic structure with hyperechoic wall and the hepatic vein as anechoic tubular structure with anechoic wall within the hepatic parenchyma. The caudal vena cava was observed as a triangular anechoic structure in transverse view and tubular in longitudinal view. It is concluded that the ultrasonography is a useful tool for the non-invasive examination of liver. Its sonographic appearance and parameters measured in healthy buffaloes can serve as reference values for the diagnosis of pathological changes in liver.

Keywords: *Bubalus bubalis*, buffaloes, hepatobiliary, biochemical studies, ultrasonography

INTRODUCTION

Though hepatic diseases are of great importance in farm animals; in bovines the signs of liver dysfunction, such as icterus or bilirubinuria have been reported to be observed less (Kremer *et al*., 1994) and the common laboratory tests for liver function also usually fail to reveal a disorder as long as one-third of the liver parenchyma remains functional (Dirksen, 1979). The diagnosis of hepatic disorders in many cases, is also reported to be difficult due to their nonspecific signs and non-availability of liver specific enzyme tests (Braun, 2009a; 2009b).

Ultrasonography has been used for the routine diagnostic procedure for many decades in canines (Nyland *et al*., 1989), and equines (Rantanen, 1986). In spite of its broader diagnostic utility, its applications in bovine ultrasonography have largely remained limited to reproductive

Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Uttar Pradesh, India, *E-mail: ishitagulshan@rediffmail.com

applications like examination of utero-tubal structures, ovaries and pregnancy diagnosis. Even in developed countries due to lack of extensive research work, the non-reproductive ultrasonographic diagnostic applications for bovines have generally remained restricted to referral clinics (Imran, 2010).

Introduction of abdominal ultrasonography in large animal diagnostic imaging has paved way for timely diagnosis of some of the conditions, more so about intra-abdominal parenchymatous organs. A complete ultrasonographic examination of the liver is reported to yield information of a high diagnostic value about the parenchymal pattern of the liver, hepatic vein (HV), portal vein (PV), caudal vena cava (CVC), size and position of the gall bladder (GB) and biliary system in cattle (Braun, 1990; Imran, 2010) as well as in buffaloes (Khalphallah *et al.*, 2016). Sonographic examination is also advised to be performed prior to more invasive procedures like liver biopsy to form a diagnosis of changes in the hepatic parenchyma (Acorda *et al.*, 1994). In the perused literature, it has been observed that there is paucity of literature on this aspect in our country and more so in buffaloes, hence it was decided to take up the present work aimed at recording ultrasonographic, Doppler study and some biochemical observations of hepatobiliary system on healthy buffaloes.

MATERIALS AND METHODS

This study was conducted on six adult apparently healthy non gravid female buffaloes, not showing any sign of systemic ailments and with normal physiological parameters presented to the clinics for procedures like artificial insemination, medial patellar desmotomy, and management of tail and horn injuries.

All buffaloes underwent a thorough clinical examination described by Jackson and Cockcroft (2008). The blood samples were collected to obtain serum for biochemical parameters analysis, in 5 ml test tubes, without anticoagulant. The serum was then stored in a deep freeze at -20^{o}C in collection tubes, duly capped and labeled with case details till analysis. Batch analysis was done to avoid the repeated thaw and freeze cycles.

The serum was then analyzed with the help of ERBA CHEM-7 BIO chemistry analyzer to estimate the serum biochemical values of Aspartate transaminase (AST), Alanine transaminase (ALT), Gamma-glutamyl tranferase (GGT) and Alkaline Phospahatase (ALP). All kits and reagents were obtained from Span diagnostics.

B mode ultrasonography of liver and gall bladder

The examination protocol of Braun (1990) was followed for B mode ultrasonographic examination of the liver. It was carried out in standing animals restrained in a trevis without any sedative. The area for scanning was prepared by clipping the hair and application of the coupling gel (Figure 1).

Statistical analysis

Data were analyzed using the SPSS 16.0 software. All data were presented as mean ± Standard Error (S.E.).

RESULTS AND DISCUSSIONS

Clinical findings

The mean values of rectal temperature, respiratory rate, heart rate and pulse rate recorded were 101.23±0.19°F, 24.66±0.71 breaths per minute, 62.16±1.16 beat per minute and 63.5±0.99 beat per minute, respectively. All animals were clinically healthy without any signs of weakness, abnormal appetite, concurrent complaint, sudden alteration in body weight, and variation in milk yield. The conjunctivae were normal pink. The pre scapular and pre femoral lymph nodes were in normal sizes on palpation.

Liver specific enzyme analysis

The mean values of AST, GGT, ALT and ALP were found to be 94.76±3.18 (U/L), 21.23±3.16 (U/L), 36.47±3.29 (U/L) and 116.23±4.79 (U/L), respectively. The serum biochemical parameters were within the normal reference range as reported by Patel *et al.* (2016) in Banni buffaloes and higher than the mean values reported by Khalphallah *et al.* (2016) in Egyptian buffaloes.

Ultrasonographic findings B-mode ultrasonography

The hepatic parenchyma was homogenously coarse echogenic, interspersed with anechoic bands of hepatic vessels, with sharp margin and was hyperechoic as compared to the right renal cortex. It was imaged from just behind the last rib to the 6th intercostal space (ICS). The lung appeared to superimpose over the liver at the level of 10th to 6th ICS on dorsal margin of the liver. The the right kidney was visualized in the hepato-renal impression at the 12th ICS or behind the last rib. At the ventral margin of the liver a part of omasum was visible from 11th to 7th intercostal space a part of intestine was visible from 11th and 12th intercostal space (Figure 2). The findings of the present study were similar to those reported by Khalphallah *et al.* (2016) in Egyptian buffaloes and by Abdelaal *et al.* (2019) in buffaloes. Abdelaal *et al.* (2019) reported that liver was imaged from the 12th and 7th ICS where Khalphallah *et al.* (2016) reported that liver was imaged in the last three intercostal spaces and caudal to costal arch while Streeter and Step (2007) in healthy cows have reported that liver can be imaged from the fifth to twelfth intercostal space and just caudal to the thirteenth rib. In the present study, liver was imaged from just behind the last rib to the 6th ICS.

The mean distance of dorsal visible margin of the liver from the dorsal midline at 12th, 11th and 10th ICS were 11±1.06 cm, 13.5±2.60 cm and 23.5±3.25 cm, respectively. Similarly, the mean distance of ventral visible margin of liver from the dorsal midline at 12th, 11th and 10th ICS were 35.5±3.12 cm, 47.5±2.63 cm and 52.66±2.29 cm respectively. No reference, of the mean values of ultrasonographic measurement of the distance of the dorsal visible margin and the ventral visible margin of liver from the dorsal midline at 12th, 11th and 10th ICS in buffaloes, could be traced in the literature reviewed (Table 1).

The mean of size of the liver at 12th, 11th and 10th ICS were 24.50 cm, 34 cm and 29.16 cm respectively (Table 1). The mean values of size of the liver at 12th, 11th and 10th ICS were similar to the mean value reported by Abdelaal *et al.* (2019); Khalphallah *et al.* (2016). Braun and Gerber (1994) have reported that size of the liver did not differ significantly among different breeds of cattle or among those of differing ages but significant differences were noted for various body weights, milk production, and stages of gestation. So we consider that the breed and bodyweight-based differences play an important role in the determination of the size of the liver.

The gallbladder was imaged as a pear-shaped fluid-filled anechoic structure with hyperechoic wall (Figure 3). The gallbladder was echolocated between the 12th to 9th ICS. The size of the gallbladder also varied, and it was visualize at one ICS in three buffaloes and two ICS in three buffaloes. The gallbladder is situated on the visceral surface of the liver. Depending upon the degree of fullness, the gallbladder was distended beyond the ventral margin of the liver, and it was visualized adjacent to the abdominal wall. The mean wall thickness of the gall bladder was 0.3±01 cm. The echo texture of gall bladder was similar to that reported by Khalphallah *et al.* (2016) however, they have described that GB could be imaged from right 10th and 11th ICS and it was not imaged from the right 12th ICS.

The portal and hepatic veins can be seen within the liver from 12th to 9th ICS. The portal vein was visualized as a stellate, branching, anechoic structure with hyperechoic wall and the hepatic vein as anechoic tubular structure without a hyperechoic wall, within the hepatic parenchyma (Figure 4). The PV and the hepatic vein were visualized ventro-lateral to the CVC at dorsal portion of 12th ICS (Figure 5). The mean diameter of the PV at 12th ICS was 3.12±0.17 cm and the mean thickness of the liver over the PV at 12th ICS was 11.73±0.49 cm. The CVC was visualized as a triangular anechoic structure in transverse view, dorsal and medial to the portal vein, at the dorsal portion of the 12th to 11th ICS and in between the liver and kidney at the lumbo-costal junction (Figure 6) and as a tubular like structure in longitudinal view (Figure 7) at the same location. The hepatic veins were seen joining the caudal vena cava towards the liver. The mean diameter of the CVC at 12th ICS was 1.52±0.16 cm and the mean thickness of the liver over the CVC at 12th ICS was 10.82±0.40 cm. These findings

Table 1 Mean ± S.E. values of B-mode ultrasonographic measurements of distance of dorsal visible margin (cm) and ventral visible margin (cm) of the liver from the dorsal midline at 12th, 11th and 10th ICS.

Measurements (cm)	12th ICS	11th ICS	10th ICS
Dorsal visible margin	11±1.06	13.5±2.63	23.5±3.25
Ventral visible margin	35.5±3.12	47.5±2.63	52.66±2.29
Size of the liver	24.50	34	29.16

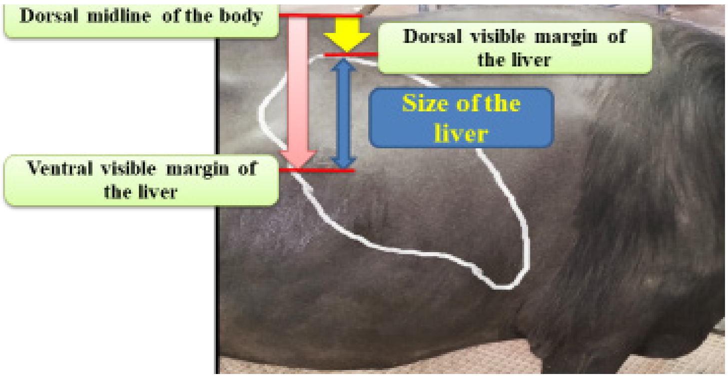

Figure 1. Schematic representation of the determination of size of the liver via ultrasonography.

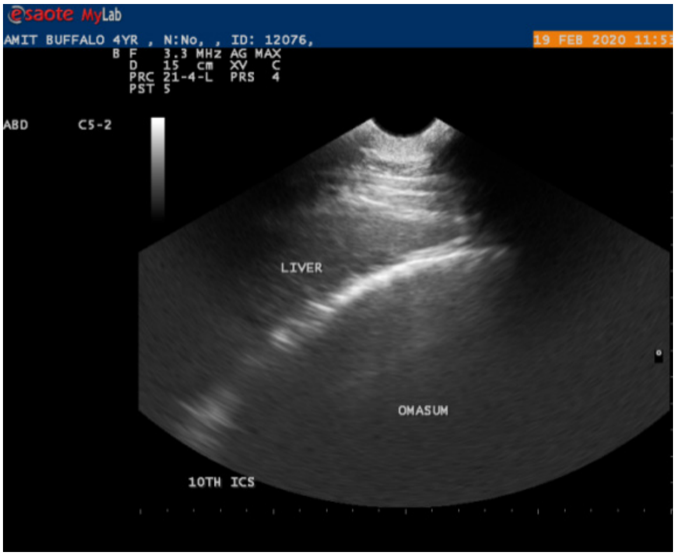

Figure 2. Sonogram of liver at 10th ICS showing homogenous coarse echogenic hepatic parenchyma, anechoic pear shaped gall baldder (GB) and half moon shaped omasual margin at ventral margin of the liver.

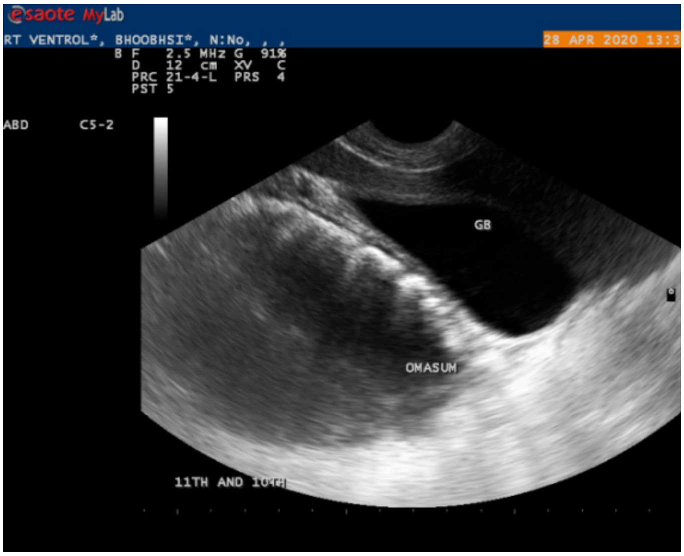

Figure 3. Sonogram of liver showing gallbladder as a pear-shaped anechoic structure with a hyperechoic wall and echogenic wall of the omasum at the ventral border of the liver.

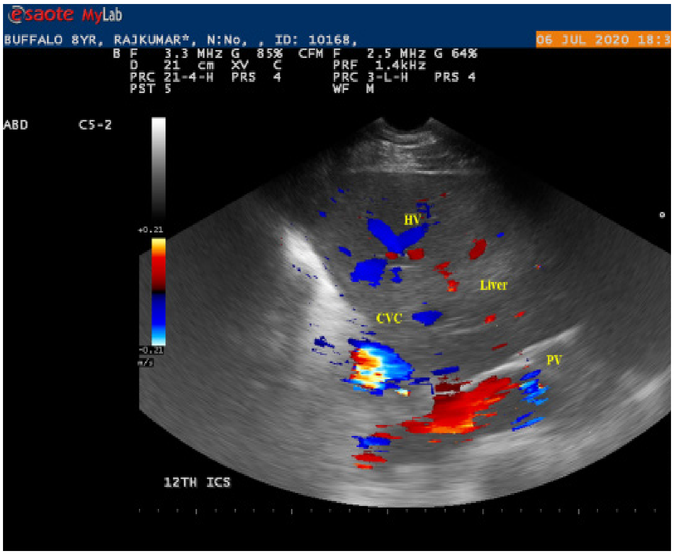

Figure 4. Sonogram of liver at 11th ICS showing portal vein as a stellate branching anechogenic structure with a hyperechogenic wall.

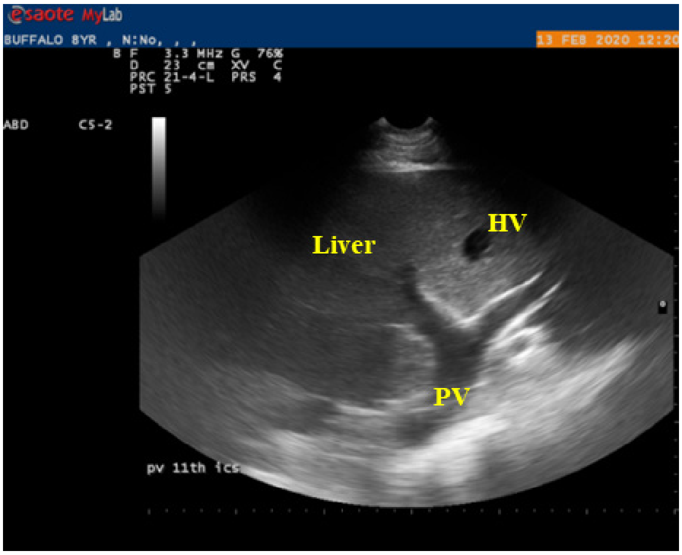

Figure 5. Doppler sonogram of liver at 11th ICS showing portal portal vein (PV) ventrolateral to the caudal vena cava (CVC) and hepatic vein (HV) in the 12th ICS.

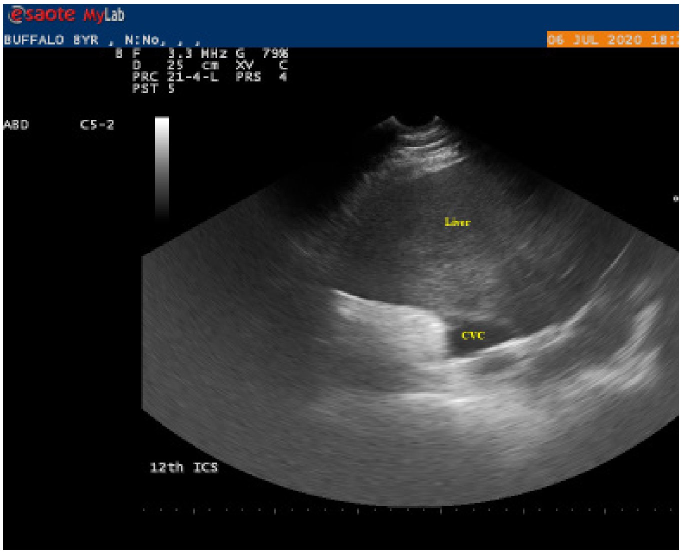

Figure 6. Sonogram of liver at dorsal portion of 12th ICS showing caudal vena cava (CVC) as a triangular anechogenic structure in transverse view.

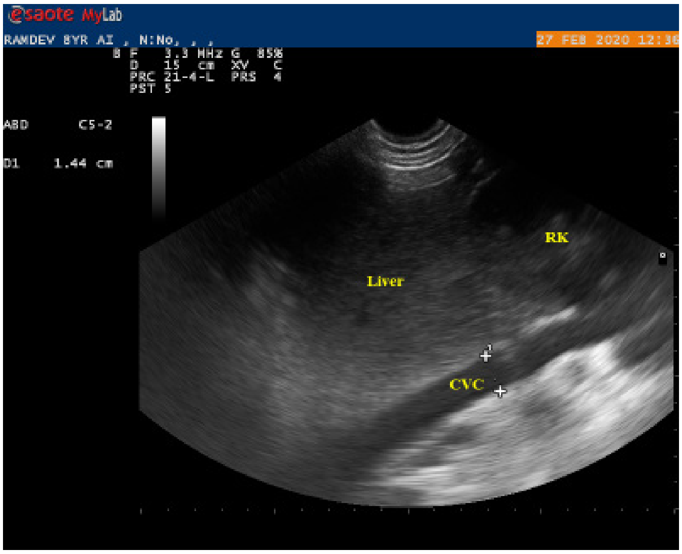

Figure 7. Sonogram of liver at dorsal portion of 12th ICS showing caudal vena cava as a tubular like structure medial to right kidney (RK) in longitudinal view.

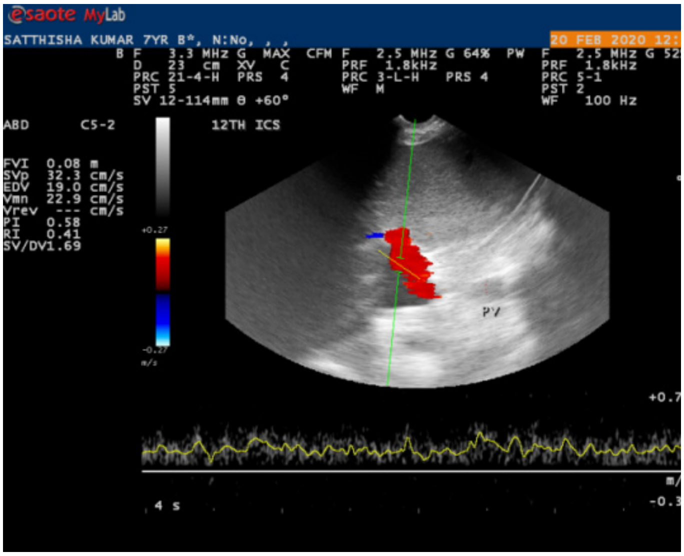

Figure 8. Spectral display showing doppler scan of PV.

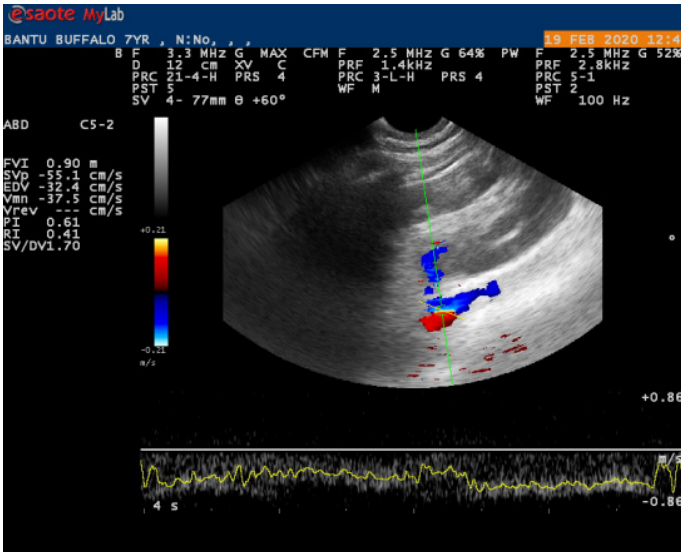

Figure 9. Spectral display showing doppler scan of CVC.

were in agreement with the Kumar *et al.* (2012) in healthy bovines; Abdelaal *et al.* (2019) in healthy buffaloes; Imran (2010) in healthy cows. The mean diameter of PV and CVC obtained in the present study were similar to that reported by Sangwan (2015) but slightly lower than that reported by Abdelaal *et al.* (2019). The mean of thickness of the liver over the PV and CVC were similar to that reported by Sangwan (2015).

Doppler ultrasonography

Spectral display of portal vein (PV) varied from turbulent flow in most of the buffaloes (Figure 8) to a laminar flow in few buffaloes. The mean values of systolic peak velocity (SVp) (cm/s), end diastolic velocity (EDV) (cm/s), mean velocity (Vmn) (cm/s), pulsatility index (PI) and resistivity index (RI) for PV were 44.32±3.16, 14.75±1.11, 21.27±3.54, 1.86±0.21 and 0.76±0.06, respectively. Spectral display of CVC showed the laminar flow (Figure 9). The mean values of SVp (cm/s), EDV (cm/s), Vmn (cm/s), PI and RI for CVC were 52.74±4.55, 19.05±3.17, 28.32±4.33, 1.66±0.56, 0.67±0.07 respectively. The mean values of SVp (cm/s), EDV (cm/s), Vmn (cm/s), PI, RI for the PV were slightly lower than the mean value reported by Sangwan (2015). Various studies in humans (Abu-Yousef, 1992), sheep (Belotta *et al.*, 2017), and cattle (Starke *et al.*, 2011; Braun and Gerber, 1994), show that the portal blood flow depends on posture, physical activity, feed intake, age, and milk yield, which also influence the size and thickness of the liver as body weight does. The mean value of SVp, EDV, Vmn (cm/s), for CVC were slightly lower than the mean value reported by Sangwan (2015). The mean values of PI and RI for CVC in the present study were slightly higher than the mean value reported by Sangwan (2015).

Braun (1990) has reported that ultrasonographic ally determined dorsoventral extents of the liver increase in cases of the end-stage congestive heart failure. In addition, the ultrasonographic appearance of the CVC is a substantial aid in diagnosing congestion in the systemic circulation. The cross-sectional image of the CVC assumes more oval to circular shape instead of triangular along with change in the CVC blood flow in the patients with impaired venous return. Whereas Verma and Swamy (2009) have discussed in detail the prevalence and pathology of hydatidosis in buffaloes, Kumar *et al.* (2016) have reported that hepatic cyst in bovines appear as single or multiple, encapsulated, thin or thick walled cavitary lesions, with anechoic to hyperechoic contents, within the hepatic parenchyma. Ahrens, (1967) has reported occurrence of hepatic abscessation after rumenitis arising from lactic acidosis accompanying grain engorgement. So these changes could be ascertained only after developing a normal baseline data in healthy animals.

From the present study it is concluded that the ultrasonography is a useful tool for the non-invasive examination of liver. Its sonographic appearance and parameters measured in healthy buffaloes can serve as reference values for the diagnosis of pathological changes in liver.

ACKNOWLEDGMENT

Authors are thankful to the Indian Council of Agricultural Research for providing financial assistance under All India Network Programme-DIMSCA.

REFERENCES

Abdelaal, A.M., E. Abd, M. Raouf, M.A. Aref and A.A. Moselhy. 2019. Clinical and ultrasonographic investigations of 30 water buffaloes (*Bubalus bubalis*) with hepatomegaly. *Vet. World*, **12**(6): 789-795. DOI: 10.14202/vetworld.2019.789-795

Abu-Yousef, M.M. 1992. Normal and respiratory variations of the hepatic and portal venous duplex doppler waveforms with simultaneous electrocardiographic correlation. *Journal of Medical Ultrasound*, **11**(6): 263-268. DOI: 10.7863/jum.1992.11.6.263

Acorda, J.A., M.H. Yamada and S.M. Ghamsari. 1994. Evaluation of fatty infiltration of the liver in dairy cattle through digital analysis of hepatic ultrasonograms. *Veterinary Radiology and Ultrasound*, **35**(2): 120-123. DOI: 10.1111/j.1740-8261.1994.tb00199.x

Ahrens, F.A. 1967. Histamine, lactic acid, and hypertonicity as factors in the development of rumenitis in cattle. *Am. J. Vet. Res.*, **28**(126): 1335-1342.

Belotta, A.F., B.P. Santarosa, D.O.L. Ferreira, S.M.F. Carvalho, R.C. Gonçalves, C.R. Padovani and M.J. Mamprim. 2017. Portal Vein Doppler flow metry in healthy sheep according to age. *Brazilian Journal of Veterinary Research and Animal Science*, **37**(10): 1172-1176. DOI: 10.1590/s0100-736x2017001000021

Braun, U. 1990. Ultrasonographic examination of the liver in cows. *Am. J. Vet. Res.*, **51**(10): 1522-1526.

Braun, U. 2009a. Ultrasonography of the gastrointestinal tract in cattle. *Vet. Clin. N. Am-Food A.*, **25**(3): 567-590. DOI: 10.1016/j.cvfa.2009.07.004

Braun, U. 2009b. Ultrasonography of the liver in cattle. *Vet. Clin. N. Am.-Food A.*, **25**(3): 591-609. DOI: 10.1016/j.cvfa.2009.07.003

Braun, U. and D. Gerber. 1994. Influence of age, breed, and stage of pregnancy on hepatic ultrasonographic findings in cows. *Am. J. Vet. Res.*, **55**(9): 1201-1205.

Dirksen, G. 1979. *Clinical Examination of Cattle*. Verlag Paul Parey, Berlin, Germany.

Imran, S. 2010. *Ultrasonography of bovine abdominal cavity*. M.V.Sc. Thesis, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya Palampur (H.P.), India.

Jackson, P. and P. Cockcroft. 2008. Cattle clinical examination by body system and region, p. 7-216. *In Clinical Examination of Farm Animals*, 1st ed. John Wiley and Sons, New Delhi, India.

Khalphallah, A., M. Abdelhakiem and E. Elmeligy. 2016. The ultrasonographic findings of the liver, gall bladder and their related vasculatures in healthy Egyptian buffaloes (*Bubalus bubalis*). *Assiut Veterinary Medical Journal*, **62**(149): 156-162. DOI: 10.21608/AVMJ.2016.169978

Kremer, H., W. Dobrinski and M.A. Schreiber. 1994. *In* Kremer, H., W. Dobrinski, I. Medizin and A. Gebiete (eds.) *Sonographic Diagnostic*, 4th ed. Urban and Schwarzenberg, Munich, Germany. 63p.

Kumar, A., N.S. Saini, J. Mohindroo and N.K. Sood. 2012. Ultrasonographic features of normal heart and liver in relation to diagnose pericarditis in bovine. *Indian J. Anim. Sci.*, **82**(12): 1489-1494.

Kumar, A., N.S. Saini, J. Mohindroo, B.B. Singh, V. Sangwan and N.K. Sood. 2016. Comparison of radiography and ultrasonography in the detection of lung and liver cysts in cattle and buffaloes. *Vet. World*, **9**(10): 1113-1120. DOI: 10.14202/vetworld.2016.1113-1120

Nyland, T.G., D.A. Hager and D.S. Herring. 1989. Sonography of the liver, gallbladder, and spleen. *Semin. Vet. Med. Surg. Small Anim.*, **4**(1): 13-31.

Patel, M.D., A. Lateef, H. Das, M.V. Prajapati, P. Kakati and H.R. Savani. 2016. Estimation of blood biochemical parameters of Banni buffalo (*Bubalus bubalis*) at different age, sex and physiological stages. *J. Livest. Sci.*, **7**: 250-255. Available on: http://livestockscience.in/wp-content/uploads/Biochem-prof-Banni-Buff.pdf

Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable. 2007. *A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 10th ed. Saunders, USA.

Rantanen, N.W. 1986. Diseases of the liver. *Vet. Clin. N. Am.-Equine*, **2**: 105-114.

Sangwan, V. 2015. *B-mode and doppler sonographic study of major blood vessels in cattle and buffalo*. Ph.D. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India.

Sood, P., A.S. Nanda, N. Singh, M. Javed and R. Prasad. 2009. Effect of lameness on follicular dynamics in crossbred cows. *Veterinary Archives*, **79**(2): 131-136. Available on: https://hrcak.srce.hr/file/58670

Starke, A., S. Schmidt, A. Haudum, T. Scholbach, P. Wohlsein, M. Beyerbach and J. Rehage. 2011. Evaluation of portal blood flow using transcutaneous and intraoperative Doppler ultrasonography in dairy cows with fatty liver. *J. Dairy Sci.*, **94**(6): 2964-2971. DOI: 10.3168/jds.2011-4156

Streeter, R.N. and D.L. Step. 2007. Diagnostic ultrasonography in ruminants. *Vet. Clin. N. Am.-Food A.*, **23**(3): 541-574. DOI: 10.1016/j.cvfa.2007.07.008

Verma, Y. and M. Swamy. 2009. Prevalence and pathology of hydatidosis in buffalo liver. *Buffalo Bull.*, **28**(4): 207-211.